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REVIEW

2nd Cancer Update

Synthesis, antimicrobial and anticancer activities of amido sulfonamido methane linked bis heterocycles

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Abstract A new class of amido sulfonamido methane linked bis heterocycles- bis-oxazoles, thiazoles and imidazoles were prepared and screened for antimicrobial and anticancer activities. The chloro substituted amido sulfonamido bisimidazole exhibited excellent antimicrobial activity and also it was the most potent compound on lung, colon and prostate cancer cell lines.

Contents

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1. Introduction

Sulfonamide drugs are associated with a wide range of biological activities and in fact brought an antibiotic revolution in medicine (Ali et al., 2006; McCarroll et al., 2007; Wilkinson et al., 2007). Many oxazole and/or thiazole containing macrocycles are naturally occurring molecules, *viz*., Bistratamides (You and Kelly, 2005), Didmolamides A and B (You and Kelly, 2005), Lyngbyabellin A (Yokokawa et al., 2001), and Calyculins (Yokokawa et al., 2001; Degnan et al., 1989; Pihko and Koskinen, 1998; Perez and Faulkner, 2003; Rudi et al., 2003; Tan et al., 2003), which show cytotoxic, antimicrobial and multiple drug resistance activities. Several classes of drugs based on imidazole *viz*., 2-nitroimidazole commonly called Azomycin are a natural antibiotic. Some synthetic nitroimidazoles are active against intestinal infections (Breccia et al., 1986). In fact metronidazole is used for intestinal infections and also as a radiosensitizer in X-ray therapy (Middlemiss and Watson, 1994). The incorporation of sulfonamide moiety into heterocyclic rings can produce pharmacologically potent compounds. The present work comprises design and synthesis of new molecules having two pharmacophoric heterocyclic units linked by bis methane amido sulfonamido moiety which are expected to have pharmacological activity. Although chemically unrelated to these compounds, other classes of antibiotics such as the anthracyclines (Miller and Stoodley, 2011) which were originally isolated from strains of *Streptomyces peucetius*, show antibacterial activity (mostly against Gram positive bacteria, for example *Staphylococcus aureus*) and have been in clinical use for the treatment of various forms of cancer for several decades. Triazolopyrazole thiones also exhibit antibacterial, antifungal and promising anticancer activities; the latter compared with the anthracycline doxorubicin.

2. Experimental

2.1. General

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The purity of the compounds was checked by TLC (silica gel H, BDH, ethyl acetate/hexane, 1:3). The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wavenumbers were given in cm^{-1}. The ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker spectrospin operating at 400 MHz. The ^{13}C NMR spectra were recorded in DMSO-d_6 on Bruker spectrospin operating at 100 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. For anticancer activity the optical density was determined at 450 nm using a microplate reader (BioTek Instruments Inc., Winooski, VT, USA).

2.2. Synthesis of bis(carbethoxymethylsulfonyl)amine (2)

To a solution of ethyl sulfamylacetate (**1**) (0.003 mol) in dichloromethane (10 ml), triethylamine (0.0031 mol), and 4-dimethylaminopyridine (DMAP) (0.0001 mol) were added and stirred at room temperature for 15 min. Then, a solution of ethyl 2-chlorosulfonylacetate (0.0033 mol) in dichlorometh-

ane (5 ml) was added dropwise and the reaction mixture was stirred at 40 °C under nitrogen atmosphere for 6–10 h. After completion of reaction the solvent was removed *in vacuo*. The resultant residue was neutralized with saturated $NaHCO_3$ solution and the aqueous layer was extracted with ethyl acetate (3×15 ml), washed with water (3×20 ml), and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residual solid was purified by column chromatography (silica gel, 60–120 mesh) using hexane-ethyl acetate (3:1) as eluent.

Yield 72%, m.p. 132–134 °C. IR (KBr): t = 3370 (NH), 1730 (C═O), 1320, 1136 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C, TMS): δ = 1.36 (t, 6H, CH_3), 4.20 (q, 4H, OCH_2), 4.42 (s, 4H, CH_2), 8.01 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 14.2 (*C*H_3), 55.8 (*C*H_2), 61.4 (O*C*H_2), 161.5 (*C*═O) ppm. Anal. For $C_8H_{15}NO_8S_2$ (317.35) cacld. C30.27, H4.76, N 4.41. Found C30.32, H4.74, N4.47.

*2.3. Synthesis of bis(carboxymethylsulfonyl)amine (**3**)*

A mixture of bis(carbethoxymethylsulfonyl)amine (**2**) (0.0025 mol), KOH (0.01 mol) in methanol (10 ml) and water (25 ml) was refluxed for 2–3 h. To this charcoal was added, boiled for 5 min. and filtered through celite. The filtrate was acidified with dil. HCl and extracted with ether. Removal of the solvent on a rotary evaporator resulted in compound **3**.

Yield 80%, m.p. 145–147 °C. IR (KBr): t = 3372 (NH), 3355 (OH), 1716 (C═O), 1323, 1141 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C, TMS): δ = 4.54 (s, 4H, CH_2), 7.96 (bs, 1H, NH), 10.43 (bs, 2H, OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 57.6 (*C*H_2), 174.2 (*C*═O) ppm. Anal. calcd For $C_4H_7NO_8S_2$ (261.24) calcd. C18.39, H2.70, N5.36. Found: C18.46, H2.71, N5.44.

*2.4. General procedure for the synthesis of bis(N-(4-aryloxazol-2-yl)aminocarbonyl–methylsulfonyl)amine (7a–c)/bis(N-(4-arylthiazol-2-yl)aminocarbonyl–methylsulfonyl)amine (8a–c)/bis(N-(4-aryl-1H-imidazol-2-yl)aminocarbonyl–methylsulfonyl)amine (**9a–c**)*

To a solution of compound **3** (0.001 mol) in dioxane (20 ml), compound **4**/**5**/**6** (0.002 mol) and 4-dimethylaminopyridine (DMAP) (0.0001 mol) were added. Then dicyclohexylcarbodiimide (DCC) (0.0014 mol) in dioxane (10 ml) was added dropwise to the contents while stirring at room temperature and continued the stirring for another 20–24 h. The separated precipitate, a dicyclohexylurea was removed by filtration. The solution was evaporated to dryness and the residual solid was purified by column chromatography (silica gel, 60–120 mesh) using hexane-ethyl acetate (3:1) as eluent.

*2.4.1. Bis(N-(4-phenyloxazol-2-yl)aminocarbonylmethylsulfonyl)amine (**7a**)*

Yield 65%, m.p. 186–187 °C. IR (KBr): t = 3344 (NH), 1690 (C═O), 1634 (C═C), 1574 (C═N), 1326, 1138 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C, TMS) δ = 4.52 (s, 4H, CH_2), 7.44–7.88 (m, 12H, Ar-H and C_5-H), 8.03 (bs, 1H, SO_2NH), 8.46 (bs, 2H, CO-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 56.2 (*C*H_2), 128.9, 129.1, 130.0, 132.6 (Ar-*C*), 138.2 (*C*-5), 140.3 (*C*-4), 149.7 (*C*-2), 167.2 (*C*═O) ppm. Anal calcd For $C_{22}H_{19}N_5O_8S_2$ (545.55) calcd. C48.43, H3.51, N12.83. Found: C48.39, H3.54, N12.93.

*2.4.2. Bis(N-(4-p-tolyloxazol-2-yl)aminocarbonylmethylsulfonyl)amine (**7b**)*

Yield 67%, m.p. 172–174 °C. IR (KBr): t = 3338 (NH), 1683 (C═O), 1630 (C═C), 1580 (C═N), 1324, 1135 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C, TMS) δ = 2.40 (s, 6H, Ar-CH_3), 4.48 (s, 4H, CH_2), 7.15-7.48 (m, 10H, Ar-H and C_5-H), 8.05 (bs, 1H, SO_2NH), 8.43 (bs, 2H, CO-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 24.8 (Ar-*C*H_3), 56.0 (*C*H_2), 127.3, 129.4, 131.0, 137.2 (Ar-*C*), 137.8 (C-5), 139.5 (C-4), 148.4 (C-2), 167.2 (*C*═O) ppm. Anal. For $C_{24}H_{23}N_5O_8S_2$ (573.60) calcd. C50.25, H, 4.04, N12.20. Found: C50.30, H4.03, N12.29.

*2.4.3. Bis(N-(4-p-chlorophenyloxazol-2-yl)aminocarbonylmethylsulfonyl)amine (**7c**)*

Yield 70%, mp 201–202 °C. IR (KBr): t = 3350 (NH), 1694 (C═O), 1627 (C═C), 1568 (C═N), 1332, 1145 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C, TMS) δ = 4.45 (s, 4H, CH_2), 7.37–7.66 (m, 10H, Ar-H and C_5-H), 7.98 (bs, 1H, SO_2NH), 8.48 (bs, 2H, CO-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 56.5 (*C*H_2), 127.5, 129.1, 132.4, 135.8 (Ar-*C*), 138.5 (C-5), 140.8 (C-4), 150.2 (C-2), 167.9 (*C*═O) ppm. Anal. For $C_{22}H_{17}Cl_2N_5O_8S_2$ (614.44) calcd. C43.00, H2.78, N11.40. Found: C43.07, H2.80, N11.48.

*2.4.4. Bis(N-(4-phenylthiazol-2-yl)aminocarbonylmethylsulfonyl)amine (**8a**)*

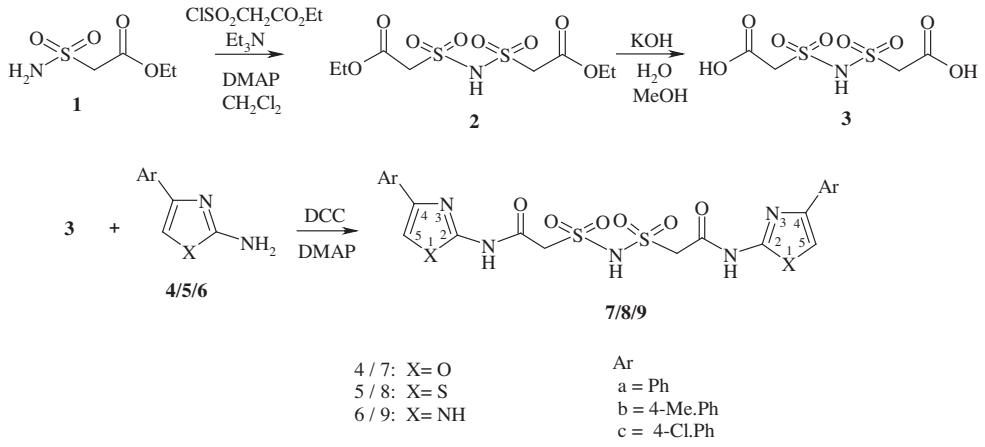
Yield 72%, m.p. 190–192 °C. IR (KBr): t = 3355 (NH), 1685 (C═O), 1632 (C═C), 1570 (C═N), 1322, 1140 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C, TMS) δ = 4.45 (s, 4H, CH_2), 7.20–7.56 (m, 12H, Ar-H and C_5-H), 7.94 (bs, 1H, SO_2NH), 8.42 (bs, 2H, CO-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 55.9 (*C*H_2), 103.7 (*C*-5), 126.7, 127.6, 128.8, 132.0 (Ar-*C*), 148.0 (*C*-4), 162.8 (*C*-2), 167.4 (*C*═O) ppm. Anal. For $C_{22}H_{19}N_5O_6S_4$ (577.68) calcd. C45.74, H3.31, N12.12. Found: C45.79, H3.30, N12.03.

*2.4.5. Bis(N-(4-p-tolylthiazol-2-yl)aminocarbonylmethylsulfonyl)amine (**8b**)*

Yield 69%, m.p. 183–185 °C. IR (KBr): t = 3348 (NH), 1673 (C═O), 1641 (C═C), 1565 (C═N), 1326, 1137 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C, TMS) δ = 2.38 (s, 6H, Ar-CH_3), 4.43 (s, 4H, CH_2), 7.10-7.43 (m, 10H, Ar-H and C_5-H), 8.01 (bs, 1H, SO_2NH), 8.39 (bs, 2H, CO-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 24.5 (Ar-*C*H_3), 55.3 (*C*H_2), 103.0 (*C*-5), 127.9, 129.5, 130.6, 136.8 (Ar-*C*), 147.7 (*C*-4), 162.1 (*C*-2), 166.6 (*C*═O) ppm. Anal. For $C_{24}H_{23}N_5O_6S_4$ (605.73) calcd. C47.58, H3.82, N11.56. Found: C47.54, H3.85, N11.66.

*2.4.6. Bis(N-(4-p-chlorophenylthiazol-2-yl)aminocarbonylmethylsulfonyl)amine (**8c**)*

Yield 75%, m.p. 215–217 °C. IR (KBr): t = 3368 (NH), 1687 (C═O), 1631 (C═C), 1575 (C═N), 1328, 1143 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C, TMS) δ = 4.38 (s,



Scheme 1 Synthesis of amido sulfonamido methane linked bis heterocycles.

4H, CH_2), 7.26-7.60 (m, 10H, Ar-*H* and C_5-*H*), 8.02 (bs, 1H, SO_2N*H*), 8.50 (bs, 2H, CO-N*H*) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 56.2 (*C*H_2), 104.2 (*C*-5), 127.8, 129.0, 131.4, 135.0 (Ar-*C*), 148.4 (*C*-4), 163.2 (*C*-2), 167.8 (*C*═O), Anal. For $C_{22}H_{17}Cl_2N_5O_6S_4$ (646.57) calcd. C40.86, H2.65, N10.83. Found: C40.90, H2.63, N10.90.

2.4.7. Bis(N-(4-phenyl-1H-imidazol-2-yl) aminocarbonylmethylsulfonyl)amine (**9a**)

Yield 77%, m.p. 224–226 °C. IR (KBr): t = 3372 (NH), 1665 (C═O), 1643 (C═C), 1560 (C═N), 1330, 1140 (SO_2) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS) δ = 4.40 (s, 4H, CH_2), 7.15–7.50 (m, 12H, Ar-*H* and C_5-*H*), 8.04 (bs, 1H, SO_2N*H*), 8.38 (bs, 2H, CO-N*H*), 11.35 (bs, 2H, N*H*) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 55.0 (*C*H_2), 120.3 (*C*-5), 127.4, 128.2, 129.7, 132.0 (Ar-*C*), 137.5 (*C*-2), 140.0 (*C*-4), 166.4 (*C*═O) ppm. Anal. For $C_{22}H_{21}N_7O_6S_2$ (543.59) calcd. C48.61, H3.89, N18.03. Found: C48.68, H3.90, N18.14.

2.4.8. Bis(N-(4-p-tolyl-1H-imidazol-2-yl) aminocarbonylmethylsulfonyl)amine (**9b**)

Yield 74%, m.p. 198–200 °C. IR (KBr): t = 3370 (NH), 1660 (C═O), 1638 (C═C), 1550 (C═N), 1325, 1130 (SO_2) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS) δ = 2.36 (s, 6H, Ar-CH_3), 4.38 (s, 4H, CH_2), 7.09–7.40 (m, 10H, Ar-*H* and C_5-*H*), 7.96 (bs, 1H, SO_2N*H*), 8.35 (bs, 2H, CO-N*H*), 11.28 (bs, 2H, N*H*) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 24.7 (Ar-*C*H_3), 54.7 (*C*H_2), 120.1 (*C*-5), 127.7, 129.6, 130.8, 135.4 (Ar-*C*), 137.1 (*C*-2), 139.7 (*C*-4), 166.0 (*C*═O) ppm. Anal. For $C_{24}H_{25}N_7O_6S_2$ (571.64) calcd. C50.42, H4.40, N17.15. Found: C50.49, H4.44, N17.24.

2.4.9. Bis(N-(4-p-chlorophenyl-1H-imidazol-2-yl) aminocarbonylmethylsulfonyl)amine (**9c**)

Yield 78%, m.p. 243–245 °C. IR (KBr): t = 3375 (NH), 1673 (C═O), 1640 (C═C), 1562 (C═N), 1335, 1144 (SO_2). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS) δ = 4.42 (s, 4H, CH_2), 7.20-7.55 (m, 10H, Ar-*H* and C_5-*H*), 7.99 (bs, 1H, SO_2N*H*), 8.40 (bs, 2H, CO-N*H*), 11.40 (bs, 2H, N*H*) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ = 55.8 (*C*H_2), 120.7 (*C*-5), 128.9, 130.1, 130.9, 135.5 (Ar-*C*), 137.9 (*C*-2), 140.4 (*C*-4), 166.8 (*C*═O) ppm. Anal. For $C_{22}H_{19}Cl_2N_7O_6S_2$ (612.48) calcd. C43.14, H3.12, N16.00. Found: C43.20, H3.11, N16.08.

2.5. Antimicrobial activity

The compounds **3**–**9** were evaluated for antimicrobial activity by the agar well diffusion method and broth dilution methods.

2.5.1. Microbial cultures

Bacterial strains *S. aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and fungi *Aspergillus niger* and *Penicillium chrysogenum* were obtained from the Department of Microbiology, S.V University, Tirupati, India.

2.5.2. Antibacterial and antifungal assays

The *in vitro* antimicrobial studies were carried out by the agar well diffusion method against test organisms (Chung et al., 1990; Azoro, 2002). Nutrient broth (NB) plates were swabbed with 24 h old broth culture (100 µl) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each petriplate. The compounds were dissolved in DMSO of 5 mg/ml and from this 10 and 20 µL (50, 100 µg/well) were added into the wells by using sterile pipettes. The standard antibiotics, Chloramphenicol, for antibacterial activity and Ketoconazole, for antifungal activity (as positive control) were simultaneously tested against the pathogens. The samples were dissolved in DMSO which showed no zone of inhibition acts as a negative control. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. After appropriate incubation, the diameter of zone of inhibition of each well was measured. Duplicates were maintained and the average values were calculated for eventual antibacterial activity.

2.5.3. Minimum inhibitory concentration assay

Broth dilution test was used to determine Minimum Inhibitory Concentration (MIC) of the above mentioned samples (Janovska et al., 2003; Bishnu et al., 2009). Freshly prepared nutrient broth was used as diluents. The 24 h old culture of the test bacteria *S. aureus*, *B. subtilis*, *P. aeruginosa*, *K. pneumoniae* and the test fungi *A. niger* and *P. chrysogenum* were diluted 100 fold in nutrient broth (100 µl bacterial

Table 1 The *in-vitro* antibacterial activity of compounds **3–9** by agar well diffusion method.

Compound	Concentration (μg/Well)	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	*B. subtilis*	*P. aeruginosa*	*K. pneumoniae*
3	50	18	20	17	16
	100	21	22	21	19
4a	50	–	–	–	–
	100	–	–	10	–
4b	50	–	–	–	–
	100	–	–	–	–
4c	50	–	–	9	–
	100	–	–	12	10
5a	50	8	–	9	9
	100	10	9	12	–
5b	50	–	–	8	–
	100	–	–	10	
5c	50	10	8	12	10
	100	13	11	16	12
6a	50	14	13	12	10
	100	17	15	16	13
6b	50	9	8	9	8
	100	11	12	11	10
6c	50	19	20	17	15
	100	21	23	19	18
7a	50	–	–	12	10
	100	–	–	15	12
7b	50	–	–	–	–
	100	–	–	10	10
7c	50	10	12	14	13
	100	13	14	15	15
8a	50	16	15	15	12
	100	18	18	19	16
8b	50	13	12	14	13
	100	15	15	17	16
8c	50	17	16	17	15
	100	19	17	20	18
9a	50	22	23	20	20
	100	24	28	24	22
9b	50	20	19	18	17
	100	23	20	22	20
9c	50	25	34	28	36
	100	30	37	32	38
Chloramphenicol	50	33	34	27	40
	100	35	38	30	42
Control (DMSO)		–	–	–	–

(–) No activity.

cultures in 10 ml NB). The stock solution of the synthesized compounds was prepared in dimethyl sulfoxide (DMSO) by dissolving 5 mg of the compound in 1 ml of DMSO. Increasing concentrations of the test samples (1.25, 2.5, 5, 10, 20, and 40 μl of stock solution contains 6.25, 12.5, 25, 50, 100, and 200 μg of the compounds) were added to the test tubes containing the bacterial and fungal cultures. All the tubes were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The tubes were examined for visible turbidity and using NB as control. Control without test samples and with solvent was assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC.

2.5.4. Minimum bactericidal/fungicidal concentration

To determine the minimum bactericidal concentration (MBC) (NCCLS publication M7-A3; Villanova, PA, 1993) and Minimum Fungicidal Concentration (MFC) (NCCLS Document M27-P; Villanova, PA, 1992) for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient broth (for bacteria) and PDA (for fungi) by streaking. Plates inoculated with bacteria and fungi were incubated at 37 °C for 24 h and at 28 °C for 48 h, respectively. After incubation, the lowest concentration was noted as MBC (for bacteria) or MFC (for fungi) at which no visible growth was observed.

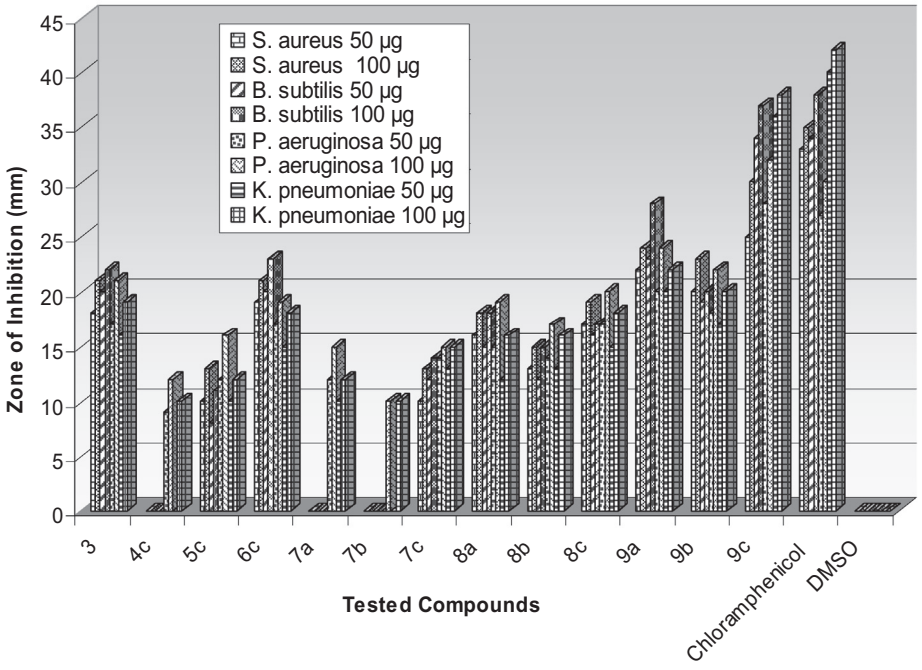


Figure 1 Antibacterial activity of **7**–**9**.

2.6. Anticancer assays

2.6.1. Compounds

The compounds **7c**, **8c**, **9a**, **9b**, and **9c** were screened for anticancer activity against NCI-H1299 (Human non-small lung cancer cells; ATCC, Manassas, VA, USA), HCT-166 p53 (Human colorectal adenocarcinoma; ATCC, Manassas, VA, USA), and PC-3 (Human prostate cancer cells; ATCC, Manassas, VA, USA) cells by EZ-cytox cell viability assay kit.

2.6.2. Cell cultures

NCI-H1299 (Human non-small lung cancer cells; ATCC, Manassas, VA, USA), and HCT-166 p53 (Human colorectal adenocarcinoma; ATCC, Manassas, VA, USA), cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) and PC-3 (Human prostate cancer cells; ATCC, Manassas, VA, USA) cells were cultured in Roswell Park Memorial Institute Medium-1640 (RPMI-1640) (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS), penicillin 100 U/ml, streptomycin 100 µg/ml, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid (HEPES) 8 mM, and l-glutamine 2 mM. Cells were maintained at 37 °C in a humidified 5% CO_2 incubator.

2.6.3. Measurement of cancer cell viability

Cell viability and proliferation were determined with EZ-cytox cell viability assay kit (Daeil Labservice, Korea) based on the cleavage of the tetrazolium salt to water-soluble formazan by succinate-tetrazolium reductase system, which belongs to the respiratory chain of the mitochondria and is active only in the viable cells. Therefore the amount of formazan dye increased with an increase in cell viability (Kwon et al., 2010). Initially, the cells were seeded into 96-well culture plates at 1×10^4 cells/ml and NCI-H1299 and HCT-166 p53 cells were cultured in DMEM and PC-3 cells were cultured in RPMI-1640 media containing 10% FBS at 37 °C. When cells reached 70% confluence, the medium was replaced with DMEM or RPMI-1640 containing 10% FBS and each 100 µM of compounds for 24 h. EZ-cytox cell viability kit reagents were added to the medium, and the cells were incubated for 1 h. The index of cell viability was determined by measuring formazan production with a microplate reader at an absorbance of 450 nm. The % cell viability was calculated by the formula:

$$\%\ \text{Cell viability} = \frac{\text{Mean absorbance in test wells}}{\text{Mean absorbance in control wells}} \times 100$$

As the % cell viability decreases the % inhibition increases. The % inhibition was calculated by the formula:

$$\%\ \text{Inhibition} = 100 - \%\ \text{cell viability}.$$

More the value of % inhibition more potent the drug is. Cells in fresh medium without any test compound were used as the control.

2.6.4. Statistical analysis

Experimental results are expressed as mean ± S.E.M. One-way ANOVA followed by Dunnett's test was used for multiple comparisons. P values of < 0.01 represent statistically significant differences.

3. Results and discussion

3.1. Chemistry

The compound ethyl sulfamylacetate (**1**) was obtained by the reaction of ethyl 2-chlorosulfonylacetate with ammonia solution (Hinman and Locatell, 1959). The compound bis(carbethoxymethylsulfonyl)amine (**2**) was prepared from

Table 2 The *in-vitro* antifungal activity of compounds **3–9** by agar well diffusion method.

Compound	Concentration (μg/Well)	Zone of inhibition (mm)	
		A. niger	*P. chrysogenum*
3	50	25	24
	100	28	28
4a	50	–	–
	100	–	–
4b	50	–	–
	100	–	12
4c	50	10	13
	100	13	15
5a	50	14	16
	100	17	18
5b	50	12	13
	100	16	16
5c	50	18	20
	100	22	23
6a	50	22	23
	100	25	27
6b	50	18	20
	100	21	24
6c	50	25	27
	100	29	31
7a	50	–	–
	100	–	–
7b	50	–	–
	100	–	–
7c	50	10	14
	100	12	17
8a	50	20	23
	100	24	26
8b	50	19	21
	100	22	24
8c	50	24	24
	100	26	28
9a	50	27	29
	100	30	34
9b	50	22	24
	100	25	26
9c	50	32	36
	100	36	39
Ketoconazole	50	33	36
	100	36	38
Control (DMSO)		–	–

(–) No activity.

ethyl 2-chlorosulfonylacetate and **1** in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP) and triethylamine (Scheme 1). The ^{1}H NMR spectrum of **2** displayed a triplet and a quartet at δ 1.36 and 4.20 for ethoxy protons, a singlet at 4.42 for methylene protons and a broad singlet at 8.01 ppm for NH. The compound **2** on hydrolysis gave bis(carboxymethylsulfonyl)amine (**3**). The absence of signals due to the ethoxy group in the ^{1}H NMR spectrum of **3** indicated that hydrolysis occurred. Besides, a broad singlet was observed at δ 10.43 ppm due to hydroxy protons in addition to signals of the methylene and NH protons. The signals due to highly acidic protons in **2** and **3** disappeared on deuteration. The compounds 4-aryloxazol-2-amine (**4**) and 4-arylthiazol-2-amine (**5**) were prepared by adopting the literature precedent from phenacyl bromide and urea/thiourea (Potewar et al., 2008). The compound 4-aryl-1*H*-imidazole-2-amine (**6**) was obtained by the reaction of phenacyl bromide with acetyl guanidine followed by hydrolysis under acidic conditions (Little and Webber, 1994). The coupling reaction of **3** with **4** in the presence of DCC and DMAP resulted in bis(*N*-(4-aryloxazol-2-yl)aminocarbonylmethylsulfonyl)amine (**7**). Similarly, the compounds bis(*N*-(4-arylthiazol-2-yl)aminocarbonylmethylsulfonyl)amine (**8**) and bis(*N*-(4-aryl-1*H*-imidazol-2-yl)aminocarbonylmethylsulfonyl)amine (**9**) were synthesized by the reaction of **3** with **5** and **6** (Scheme 1). The ^{1}H NMR spectra of **7a**, **8a** and **9a** exhibited a singlet at δ 4.52, 4.45 and 4.40 for methylene protons, two broad singlets at δ 8.03, 7.94, 8.04 and 8.46, 8.42, and 8.38 for SO_2NH and CONH, respectively. However, a singlet due to C_5-H appeared in a more downfield region, merged with aromatic protons at 7.53, 7.50 and 7.45 ppm. The structures of all the compounds were further ascertained by IR, ^{13}C NMR spectra and microanalyses.

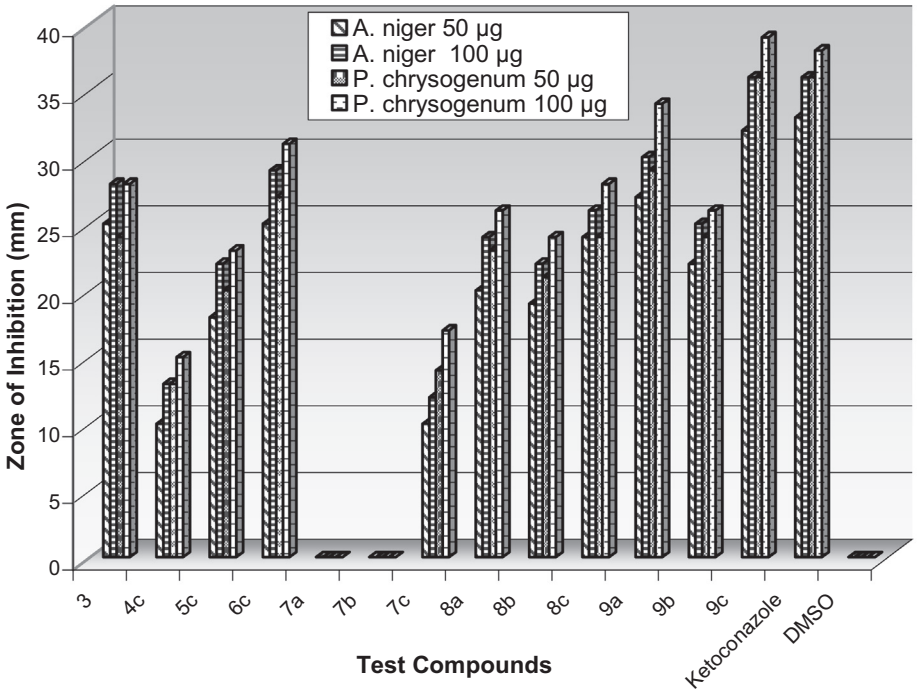


Figure 2 Antifungal activity of **7**–**9**.

Table 3 MIC, MBC and MFC of compounds **9a** and **9c** in broth dilution method.

Compound	Minimum inhibitory concentration MIC (MBC/MFC) µg					
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K. pneumoniae*	*A. niger*	*P. chrysogenum*
9a	50 (200)	25 (100)	25 (100)	100 (>200)	50 (200)	100 (>200)
9c	25 (100)	6.25 (12.5)	6.25 (12.5)	50 (200)	6.25 (12.5)	12.5 (25.0)
Chloramphenicol	6.25	6.25	6.25	12.5	–	–
Ketoconazole	–	–	–	–	6.25	12.5

S, *Staphylococcus*; B, *Bacillus*; P, *Pseudomonas*; K, *Klebsiella*; A, *Aspergillus*; P, *Penicillium*.

3.2. In-vitro antimicrobial activity

The compounds **3**–**9** were tested for antimicrobial activity by agar well diffusion and broth dilution methods. The results of antibacterial activity shown in Table 1 revealed that Gram-negative bacteria were more susceptible toward the tested compounds than Gram-positive bacteria (Fig. 1). The compound **3** exhibited moderate antibacterial activity. Among **4**, **5** and **6**, compound **6c** showed good antibacterial activity against Gram-negative bacteria. However, the compounds having amido-sulfonamido linkage **7**–**9** displayed greater activity when compared with the synthetic intermediates **3**–**6**. The compound **9c** exhibited pronounced activity particularly against *B. subtilis* and *P. aeruginosa* at 50 and 100 µg/mL when compared with the standard drug Chloramphenicol. The bis heterocycles having thiazole moiety (**8**) showed moderate activity against the tested bacteria. On the other hand, compounds with bisoxazole unit (**7**) exhibited least activity. The presence of the chloro substituent on the aromatic ring enhanced the activity. All the tested compounds inhibited the spore germination against tested fungi except the compounds **4** and **7**. It was observed that the amido-sulfonamido linked bis-heterocycles **7**–**9** showed greater antifungal activity than the compounds **3**–**6**. In fact, compound **9c** displayed higher activity than the standard drug Ketoconazole against both fungi (Table 2). All the compounds displayed slightly higher antifungal activity toward *P. chrysogenum* than *A. niger* (Fig. 2).

The MIC, MBC and MFC values of the compounds tested are listed in Table 3. The compound **9c** exhibited low MIC values when compared with **9a**. The MBC value of compound **9c** is 2× MIC in case of *B. subtilis* and *P. aeruginosa* and MFC value is 2× MIC in case of *A. niger* and *P. chrysogenum*. However, **9a** showed bactericidal and fungicidal effects greater than 2× MIC. The structure-antimicrobial activity relationship of the tested compounds indicated that the amido-sulfonamido methane linked bisimidazoles are more active than bisthiazoles and bisoxazoles. The chloro substituted bisimidazole showed strong antibacterial activity against *B. subtilis* and *P. aeruginosa* and also strong antifungal activity against *A. niger* and *P. chrysogenum*.

3.3. In-vitro anticancer activity

The amido-sulfonamido methane linked bis heterocycles **7**, **8** and **9** were screened against lung (NCI-H1299), colon

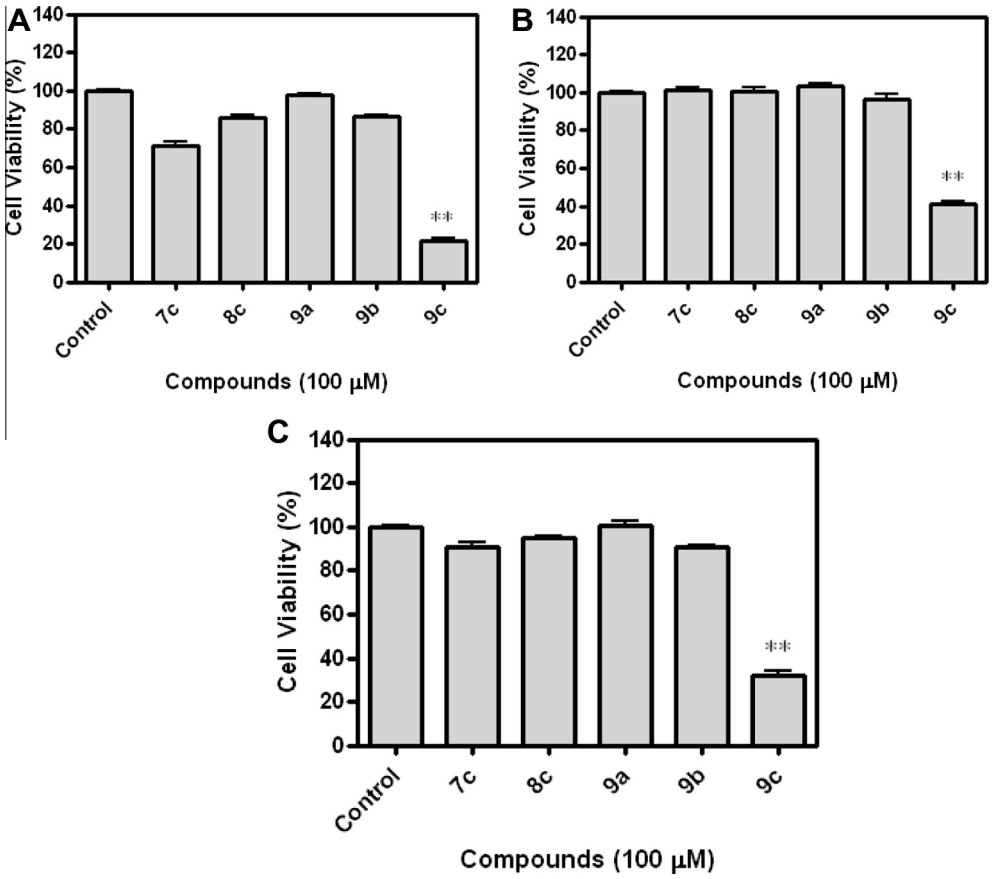


Figure 3 Effects of amido sulfonamido methane linked bis heterocycles on cancer cell lines. Cells were seeded in 96-well culture plates at 1×10^4 cells/ml NCI-H1299 and HCT-166 p53 cells were cultured in DMEM and PC-3 cells were cultured in RPMI-1640 media containing 10% FBS at 37 °C. When cells reached 70% confluence, the medium was replaced with DMEM or RPMI-1640 containing 10% FBS and 100 μM of amido sulfonamido methane linked bis heterocycles (**7c**, **8c**, **9a**, **9b** and **9c**) for 23 h. Values represent means ± S.E.M. from three different assays. (A) NCI-H1299, (B) HCT-166 p53, and (C) PC-3 cancer cell line. $^{**}p < 0.01$ compared with control.

Table 4 Anticancer activity of **7c**, **8c**, **9a**, **9b** and **9c** on NCI-H1299, HCT-166 p53 and PC-3 cell lines at 100 μM concentration. Cell viability in %.

Compound	NCI-H1299		HCT-166 p53		PC-3	
	% Viability (% inhibition)	SD	% Viability (% inhibition)	SD	% Viability (% inhibition)	SD
Control	100.00 (0)	±1.76	100.00 (0)	±2.01	100.00 (0)	±1.34
7c	70.93 (29.07)	±4.08	101.50 (−1.5)	±2.43	90.68 (9.32)	±4.76
8c	85.68 (14.32)	±3.40	100.80 (−0.8)	±3.10	95.21 (4.79)	±1.39
9a	97.45 (2.55)	±2.67	102.52 (−2.52)	±2.07	100.53 (−0.53)	±4.29
9b	86.24 (13.76)	±2.47	96.69 (3.31)	±5.64	90.95 (9.05)	±1.15
9c	21.40 (78.60)	±2.25	41.38 (58.62)	±1.78	32.09 (67.91)	±4.23

Table 5 Anticancer activity of **9c** on NCI-H1299, HCT-166 p53 and PC-3 cell lines at 0, 10, 50, and 100 μM concentrations. Cell viability in %.

Compound **9c** (μM)	NCI-H1299		HCT-166 p53		PC-3	
	% Viability (% inhibition)	SD	% Viability (% inhibition)	SD	% Viability (% inhibition)	SD
0	100.00 (0)	±3.10	100.00 (0)	±3.10	100.00 (0)	±3.10
10	98.26 (1.74)	±3.08	102.67 (−2.67)	±8.11	54.90 (45.10)	±3.00
50	19.99 (80.01)	±8.33	89.12 (10.88)	±1.43	39.38 (60.62)	±1.28
100	19.28 (80.72)	±3.60	44.00 (56.00)	±0.34	36.61 (63.39)	±3.40

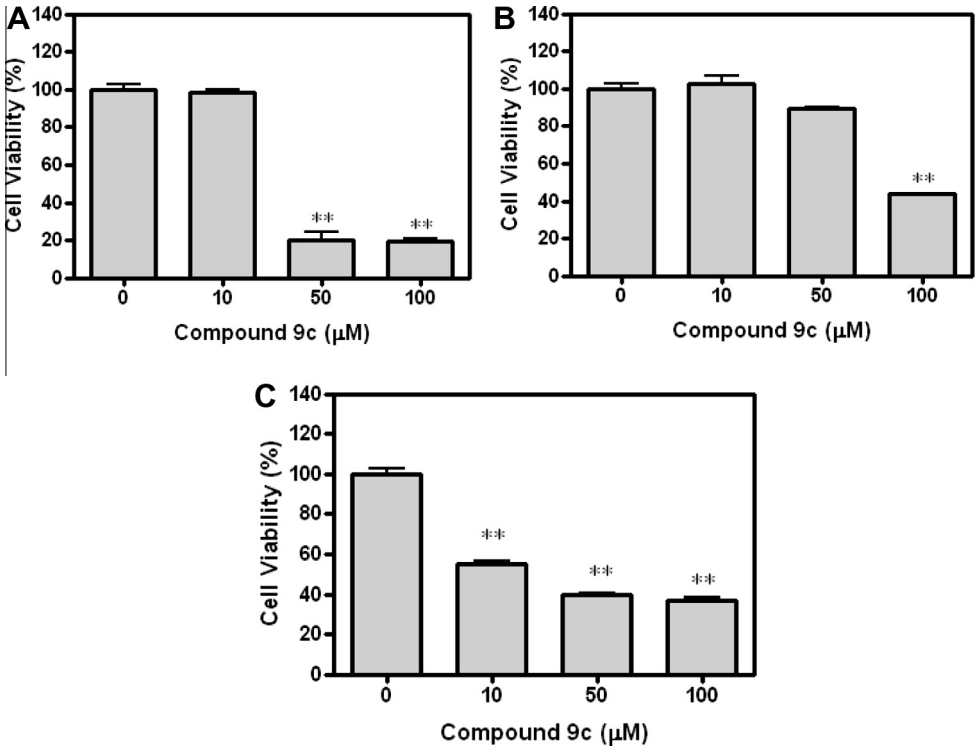


Figure 4 The effect of compound **9c** on NCI-H1299, HCT-166 p53 and PC-3 cancer cell lines: precultured, treated with compound **9c** (0–100 μM) for 23 h, EZ-cytox cell viability kit reagents were added to the medium and the cells were incubated for 1 h. The optical density was determined at 450 nm using a microplate reader. Values represent means ± S.E.M. from three different assays. (A) NCI-H1299, (B) HCT166 p53, and (C) PC-3 cancer cell line. $^{**}p < 0.01$ compared with control.

(HCT-166 p53) and prostate (PC-3) cancer cell lines by EZ-cytox cell viability assay kit. To determine the anticancer activity of bis heterocycles **7**–**9**, the cancer cells were treated at concentrations from 100 μM for 24 h and measured the cell viability using the EZ-cytox cell viability kit. As shown in Fig. 3, the evidence of the anticancer effect of compound **9c** on NCI-H1299, HCT-166 p53 and PC-3 cancer cells can be seen. The inhibition percentage of compound **9c** was 78.60 (NCI-H1299), 58.62 (HCT-166 p53) and 67.91 (PC-3) at 100 μM, respectively (Table 4 and Fig. 3). With compound **9c** (0–100 μM) stimulation for 24 h, cancer cells also decreased in a dose-dependent manner (Table 5 and Fig. 4). This result suggests that the compound **9c** pre-treatment was clearly shown to modulate the anticancer activity.

4. Conclusion

- A new class of amido-sulfonamido methane linked bis heterocycles-bisoxazoles (**7**), bisthiazoles (**8**) and bisimidazoles (**9**) were prepared from bis(carboxymethyl–sulfonyl)amine and amino-oxazoles (**4**), thiazoles (**5**) and imidazoles (**6**).
- Bis heterocycles (**7**–**9**) were found more active than the respective mono heterocycles (**4**–**6**).
- The compounds having oxazole unit **4** and **7** were found inactive against both bacteria and fungi.
- Compounds having chloro substituent showed good antimicrobial activity.
- The chloro substituted bisimidazole **9c** exhibited excellent antibacterial activity against *B. subtilis*, *P. aeruginosa* and antifungal activity against *A. niger*, and *P. chrysogenum*.
- The compound **9c** was the most potent compound on cancer cells which may potentiate cancer therapy regimens now in the development of lung, colon and prostate cancer cell lines.

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References

Ali, A., Reddy, G.S.K.K., Cao, H., Anjum, S.G., Nalam, M.N.L., Schiffer, C.A., Rana, T.M., 2006. J. Med. Chem. 49, 7342–7356.
Azoro, C., 2002. World J. Biotechnol. 3, 347–357.
Bishnu, J., Sunil, L., Anuja, S., 2009. J. Sci. Eng. Technol. 5, 143–150.
Breccia, A., Cavalleri, B., Adams, G.E., 1986. Chemistry, Pharmacology and Clinical Applications. Plenum Press, VCH, Florida.
Chung, K.T., Thomasson, W.R., Wu-Yuan, C.D., 1990. J. Appl. Bacteriol. 69, 498–503.
Degnan, B.M., Hawkins, C.J., Lavin, M.F., McCaffrey, E.J., Parry, D.L., Watters, D.J., 1989. J. Med. Chem. 32, 1354–1359.
Hinman, R.L., Locatell Jr., L., 1959. J. Am. Chem. Soc. 5, 5655–5658.
Janovska, D., Kubikova, K., Kokoska, L., 2003. J. Food Sci. 21, 107–110.
Kwon, H.K., Hwang, J.S., So, J.S., Lee, C.G., Sahoo, A., Ryu, J.H., Jeon, W.K., Ko, B.S., Im, C.R., Lee, S.H., Park, Z.Y., Im, S.H., 2010. BMC Cancer 10, 392.
Little, T.L., Webber, S.E., 1994. J. Org. Chem. 59, 7299–7305.
McCarroll, A.J., Bradshaw, T.D., Westwell, A.D., Matthews, C.S., Stevens, M.F.G., 2007. J. Med. Chem. 50, 1707–1710.
Middlemiss, D., Watson, S.P., 1994. Tetrahedron 50, 13049–13080.

Miller, J.P., Stoodley, R.J., 2011. J. Saudi Chem. Soc. 15, 275–281.
National committee for clinical laboratory standards, reference method for broth dilution antifungal susceptibility testing of yeasts, proposed standard, 1992. NCCLS Document M27-P. Villanova, PA.
National committee for clinical laboratory standards methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3d edition; approved standard, NCCLS publication M7-A3; Villanova, PA, 1993.
Perez, L.J., Faulkner, D.J., 2003. J. Nat. Prod. 66, 247–250.
Pihko, P.M., Koskinen, A.M.P., 1998. J. Org. Chem. 63, 92–98.
Potewar, T.M., Ingale, S.A., Srinivasan, K.V., 2008. Tetrahedron 64, 5019–5022.
Rudi, A., Chill, L., Aknin, M., Kashman, Y., 2003. J. Nat. Prod. 66, 575–577.
Tan, L.T., Sitachitta, N., Gerwick, W.H., 2003. J. Nat. Prod. 66, 764–771.
Wilkinson, B.L., Bomaghi, L.F., Houston, T.A., Innocenti, A., Vullo, D., Supuran, C.T., Poulsen, S.A., 2007. J. Med. Chem. 50, 1651–1657.
Yokokawa, F., Sameshima, H., Shioiri, T., 2001. Tetrahedron Lett. 42, 4171–4174.
You, S.-L., Kelly, J.W., 2005a. Tetrahedron 61, 241–249.
You, S.-L., Kelly, J.W., 2005b. Tetrahedron Lett. 46, 2567–2570.